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ORIGINAL RESEARCH

# The effect of vagal nerve blockade using electrical impulses on glucose metabolism in nondiabetic subjects

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**Purpose:** Vagal interruption causes weight loss in humans and decreases endogenous glucose production in animals. However, it is unknown if this is due to a direct effect on glucose metabolism. We sought to determine if vagal blockade using electrical impulses alters glucose metabolism in humans.

**Patients and methods:** We utilized a randomized, cross-over study design where participants were studied after 2 weeks of activation or inactivation of vagal nerve blockade (VNB). Seven obese subjects with impaired fasting glucose previously enrolled in a long-term study to examine the effect of VNB on weight took part. We used a standardized triple-tracer mixed meal to enable measurement of the rate of meal appearance, endogenous glucose production, and glucose disappearance. The 550 kcal meal was also labeled with <sup>111</sup>In-diethylene triamine pentaacetic acid (DTPA) to measure gastrointestinal transit. Insulin action and β-cell responsivity indices were estimated using the minimal model.

**Results:** Integrated glucose, insulin, and glucagon concentrations did not differ between study days. This was also reflected in a lack of effect on β-cell responsivity and insulin action. Furthermore, fasting and postprandial endogenous glucose production, integrated meal appearance, and glucose disposal did not differ in the presence or absence of VNB. Similarly, gastric emptying and colonic transit were unchanged by VNB.

**Conclusion:** In this pilot study in nondiabetic humans, electrical vagal blockade had no acute effects on glucose metabolism, insulin secretion and action, or gastric emptying. It remains to be determined if more pronounced effects would be observed in diabetic subjects.

**Keywords:** vagotomy, insulin secretion, insulin action, endogenous glucose production

## Introduction

Bilateral truncal vagotomy results in early satiety and weight loss believed to be, at least in part, due to a decrease in gastric accommodation.<sup>1,2</sup> Electrical vagal nerve blockade (VNB) for the treatment of refractory epilepsy has also been reported to cause weight loss in some,<sup>3,4</sup> but not all,<sup>5,6</sup> case series. When used to treat obesity, electrical blockade of the vagal trunks at the level of the gastric cardia results in decreased caloric intake and significant loss of excess weight in the first 6 months after implantation.<sup>7</sup> However, weight loss is not sustained 1 year after implantation.<sup>8</sup>

Following vagotomy, adaptive changes in the enteric nervous system, which may include formation of collateral innervation, result in minimal disruption of gastrointestinal function over time.<sup>9,10</sup> While animal studies suggest that vagal interruption has effects on insulin secretion,<sup>11–13</sup> insulin action,<sup>14</sup> and hepatic glucose metabolism,<sup>15</sup> it presently is unknown if these effects occur in humans following chronic vagal denervation.

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The use of chronic, but reversible,<sup>16</sup> electrical vagal blockade to treat obesity allows direct study of the effect of vagal function on postprandial carbohydrate metabolism and insulin secretion and action independent of changes in weight. In the present experiments we sought to determine if vagal blockade alters  $\beta$ -cell function and postprandial glucose metabolism. The oral and C-peptide minimal models were utilized as before.<sup>17</sup> The triple tracer method was used to measure postprandial suppression of glucose production and postprandial stimulation of glucose disposal as well as the rate of the systemic appearance of the ingested glucose.<sup>18 111</sup>In-diethylene triamine pentaacetic acid (DTPA; Mallinckrodt Pharmaceuticals, St. Louis, MO, USA) in the meal enabled measurement of gastric emptying and small intestinal transit.<sup>19</sup> Subjects were studied on two occasions in random order with VNB activated or inactivated in the 2-week period preceding each experiment. The investigators performing the assays and analyses were blinded until all studies were completed. The primary outcome was an effect of VNB on disposition index (DI) and the secondary outcome was an effect on gastric emptying – specifically the time taken to empty 50% of gastric contents.

## Materials and methods

### Subjects

After approval from the Mayo Institutional Review Board, seven nondiabetic subjects gave written, informed consent to participate in the study. These subjects had previously undergone placement of VNB devices as part of a multicenter observational study examining the long-term effects of vagal inhibition<sup>7</sup> and were weight stable. At the time of recruitment, all participants were actively using their VNB devices on a daily basis for >12 months. During the randomized phase of the trial, which ended >12 months before recruitment, subjects receiving active treatment experienced a  $17\% \pm 2\%$  excess weight loss, while those receiving sham treatment lost  $16\% \pm 2\%$  excess weight. There was no significant difference between groups.<sup>8</sup> The VNB devices have been described in detail previously – they comprise two electrodes (one for each vagal trunk) placed laparoscopically under general anesthesia, a neuroregulator placed subcutaneously, and an external controller attached to a transmit coil to communicate with and power the device.<sup>7</sup> Subjects were instructed to wear the device for a minimum of 10 hours daily. When worn, the external controller provides 5 minutes of 5,000 Hz and an amplitude of ~6 mA alternating with 5 minutes of no electrical signal. Subjects had no active illness and did not engage in regular vigorous exercise. All subjects were instructed to follow a weight maintenance diet (~55% carbohydrate,

30% fat, and 15% protein) for the period of study. Body composition was measured using dual-energy X-ray absorptiometry (DPX scanner; Lunar, Madison, WI, USA). Gastrointestinal symptoms were assessed by the Bowel Disease Questionnaire.<sup>20</sup>

### Experimental design

Subjects were studied using a labeled mixed meal and were randomized to either continue vagal blockade for 2 weeks or to discontinue vagal blockade for 2 weeks. A mixed meal study was then undertaken and subjects crossed over to resume vagal blockade in one group and discontinue blockade in the other. A mixed meal was repeated after the second 2-week period. The trial was registered at <http://www.clinicaltrials.gov> (NCT01117311).

Participants were admitted to the Clinical Research Unit at 5 pm the night before the study, consumed a standardized low-calorie mixed meal (400 Kcal: 55% carbohydrate, 30% fat, and 15% protein), after which they fasted overnight. At 6 am (~180 minutes), a forearm vein was cannulated with an 18 gauge needle to allow infusions to be performed. An 18 gauge cannula was inserted retrogradely into a vein of the dorsum of the contralateral hand. This was placed in a heated Plexiglas box (Department of Biomedical Engineering, Mayo Clinic Rochester, MN, USA) maintained at 55°C to allow sampling of arterialized venous blood. A primed (12 mg/kg) continuous (0.12 mg/kg/min) infusion of [6,6-<sup>2</sup>H]<sub>2</sub> glucose (PerkinElmer Life Sciences, Waltham, MA, USA) was initiated. At 9 am (0 minutes), subjects consumed a meal consisting of two scrambled eggs, 50 g of Canadian bacon and Jell-O containing 70 g of glucose labeled with [1-<sup>13</sup>C] (4% enrichment). This meal provided 510 kcal (61% carbohydrate, 19% protein, and 21% fat). To enable measurement of solid phase gastric emptying, the egg was labeled with 0.1 mCi <sup>111</sup>In-DTPA. An infusion of [6-<sup>3</sup>H] glucose was started at this time, and the infusion rate varied to mimic the anticipated appearance of meal [1-<sup>13</sup>C] glucose. The rate of infusion of [6,6-<sup>2</sup>H]<sub>2</sub> glucose was altered to approximate the anticipated fall in endogenous glucose production (EGP) thereby minimizing changes in specific activity.<sup>21,22</sup>

Gastrointestinal transit was measured by anterior and posterior gamma camera images obtained immediately after meal ingestion, every 15 minutes for the first 2 hours, then every 30 minutes for the next 2 hours (total 4 hours after the meal) for gastric emptying measurements. To evaluate orocecal transit, scintigraphy was repeated 6 and 24 hours after meal ingestion so as to quantitate and localize the remaining <sup>111</sup>In in the intestine using a single scan of the gamma camera. To locate the cecum, a radioisotope marker was placed on the

patient's right anterior superior iliac spine.<sup>19</sup> Overall colonic transit was analyzed using the geometric center method, where the weighted average of counts in four colonic regions (ascending, transverse, descending, and rectosigmoid) and stool was estimated by quantifying the isotopic counts in each of these regions, and correcting for isotope decay. A score of 1 reflects the average position of isotope at the hepatic flexure, 2 at the splenic flexure, 3 at the junction of the descending and sigmoid, 4 at the anal canal, and 5 in the stool.<sup>19</sup>

## Analytical techniques and glucose turnover

Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at -20°C until assayed. Glucose concentrations were measured using a glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH, USA). Plasma insulin was measured using a chemiluminescence assay (Access Assay; Beckman Coulter, Chaska, MN, USA). Plasma glucagon and C-peptide were measured by Radio-Immunoassay (EMD Millipore, Billerica, MA, USA). Plasma [6,6-<sup>2</sup>H<sub>2</sub>] glucose and [1-<sup>13</sup>C] glucose enrichments were measured using gas chromatographic mass spectrometry (Thermoquest, San Jose, CA, USA) to simultaneously monitor the C-1 and C-2 and C-3 to C-6 fragments, as described by Beylot et al.<sup>23</sup> In addition, [6-<sup>3</sup>H] glucose specific activity was measured by liquid scintillation counting following deproteinization and passage over anion and cation exchange columns.<sup>22</sup>

## Calculations

The systemic rates of meal appearance ( $R_a_{meal}$ ), EGP, and glucose disappearance were calculated using Steele's two compartment model.<sup>24</sup>  $R_a_{meal}$  was calculated by multiplying rate of appearance of [1-<sup>13</sup>C] glucose (obtained from the infusion rate of [6-<sup>3</sup>H] glucose and the clamped plasma ratio of [6-<sup>3</sup>H] glucose and [1-<sup>13</sup>C] glucose) by the meal enrichment. EGP was calculated from the infusion rate of [6,6-<sup>2</sup>H<sub>2</sub>] glucose and the ratio of [6, 6-<sup>2</sup>H<sub>2</sub>] glucose to endogenous glucose concentration. Glucose disappearance was calculated by subtracting the change in glucose mass from the overall rate of glucose appearance (ie,  $R_a_{meal}$  + EGP + infused [6,6-<sup>2</sup>H<sub>2</sub>] glucose). Values from -30 to 0 minutes were averaged and considered as basal. Area under the curve was calculated using the trapezoidal rule.

## Measurement of insulin secretion and action

Net insulin action ( $S_p$ ) was measured using the oral minimal model, which estimates insulin action from the

changes in glucose and insulin concentrations during the meal challenge.<sup>25</sup> The methodology underlying the oral minimal model has been the subject of a recent review.<sup>26</sup> Modeling the response to an oral challenge allows simultaneous measurement of insulin secretion and action, as well as the extraction of portal insulin by the liver prior to appearing in the systemic circulation.  $\beta$ -Cell responsiveness indices were estimated using the oral C-peptide minimal model, utilizing the changes in glucose and C-peptide concentrations during the meal challenge.<sup>27</sup> Insulin secretion is obtained from C-peptide concentrations by a process of deconvolution, which incorporates age-associated changes in C-peptide kinetics.<sup>28</sup> An index of total beta-cell responsivity to glucose ( $\phi_{total}$ ) is derived subsequently.<sup>29</sup> DI were calculated by multiplying  $\phi_{total}$  by  $S_p$ . Hepatic extraction was calculated from the ratio of peripheral insulin to portal insulin (as calculated from C-peptide) as previously described.<sup>30</sup>

## Statistical analysis

Data in the text are presented as means  $\pm$  standard error of the mean (SEM). The primary analysis compared data obtained during the meal study where vagal blockade was activated in the 2 weeks prior to study (VNB on) or inactivated in the 2 weeks prior to study (VNB off). A Wilcoxon matched-pairs signed rank test (two-tailed), which does not assume Gaussian distribution, was used to test the significance of the changes of study variables between study days. The statistical analysis was undertaken in Primer 5 (GraphPad Software, Inc., La Jolla, CA, USA). A  $P$ -value  $<0.05$  was considered statistically significant.

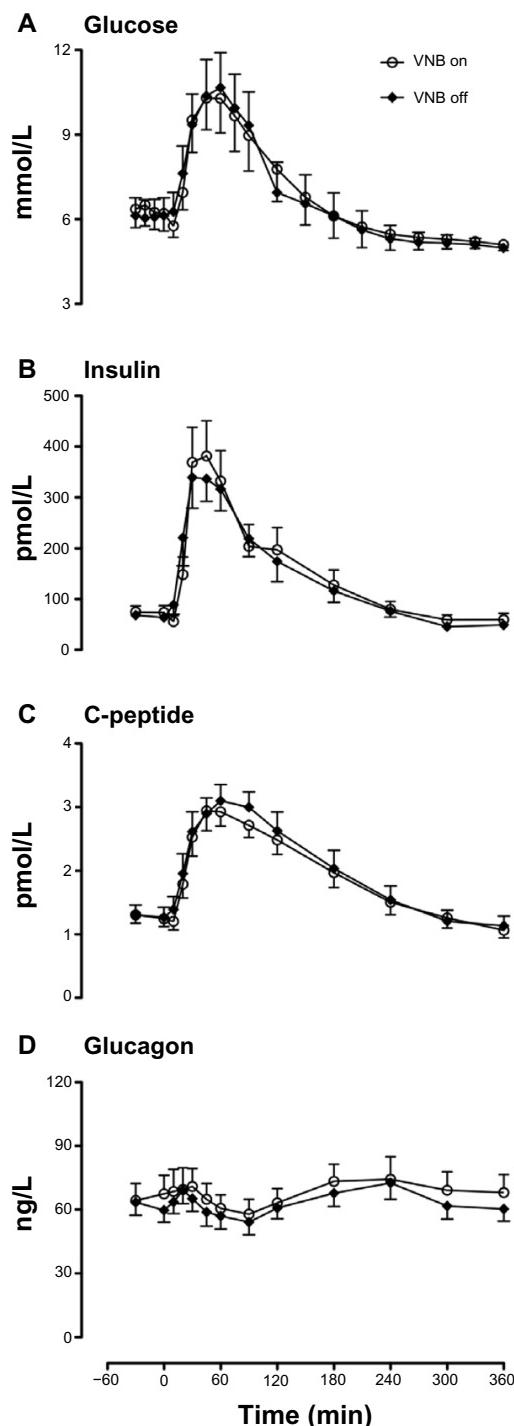
## Results

### Volunteer characteristics

Seven of nine subjects (all female) participating at Mayo Clinic, Rochester, MN or University of Minnesota, Minneapolis, MN in the observation phase of the EMPOWER study<sup>8</sup> gave written, informed consent to participate in the study. The remaining two subjects declined to participate in this study. The study participants' mean age was  $53.1 \pm 1.8$  years. At the time of screening, weight was  $104 \pm 5$  kg, body mass index (BMI) was  $37.3 \pm 1.2$  kg/m<sup>2</sup> and lean body mass was  $49 \pm 6$  kg. The VNB device was interrogated at the time of each study visit to ensure appropriate duration of use, and proper function, over the 2-week interval preceding each study. Subjects enrolled in the study were at stable weight, and did not have significant gastrointestinal symptoms as measured by the Bowel Disease Questionnaire.<sup>20</sup>

## Plasma glucose, insulin, C-peptide, and glucagon concentrations in the presence or absence of vagal nerve blockade

Fasting glucose ( $6.3 \pm 0.6$  versus [vs]  $6.1 \pm 0.6$  mmol/L,  $P=0.11$ ) just prior to meal ingestion did not differ when VNB was pres-



**Figure 1** Glucose (A), insulin (B), C-peptide (C) and glucagon (D) concentrations in the presence (white open circle) or absence of VNB (solid black diamond). Abbreviations: min, minute; VNB, vagal nerve blockade.

ent or absent, respectively (Figure 1). Postmeal peak ( $10.7 \pm 1.2$  vs  $10.9 \pm 1.2$  mmol/L,  $P=0.55$ ) and integrated postprandial glucose concentrations ( $2,430 \pm 260$  vs  $2,440 \pm 260$  mmol per 6 hours,  $P=1.00$ ) did not differ in the presence or absence of VNB (Figure 1A).

Fasting ( $74 \pm 12$  vs  $66 \pm 10$  pmol/L,  $P=0.16$ ), postmeal peak insulin concentrations ( $412 \pm 69$  vs  $426 \pm 53$  pmol/L,  $P=0.69$ ) as well as integrated postprandial insulin concentrations ( $53.4 \pm 9.0$  vs  $50.0 \pm 6.5$  nmol/L per 6 hours,  $P=0.69$ ) did not differ in the presence or absence of vagal blockade (Figure 1B).

Fasting ( $1.3 \pm 0.1$  vs  $1.3 \pm 0.2$  nmol/L,  $P=0.85$ ) and postprandial ( $3.2 \pm 0.2$  vs  $3.2 \pm 0.3$  nmol/L,  $P=0.62$ ) C-peptide concentrations (Figure 1C) and fasting ( $66 \pm 8$  vs  $62 \pm 6$  ng/L,  $P=0.25$ ), peak ( $82 \pm 9$  vs  $76 \pm 7$  ng/L,  $P=0.22$ ), and nadir ( $55 \pm 7$  vs  $53 \pm 6$  ng/L,  $P=0.85$ ) postprandial glucagon concentrations also did not differ in the presence or absence of vagal blockade (Figure 1D).

## Endogenous glucose production, meal appearance, and glucose disappearance in the presence or absence of vagal nerve blockade

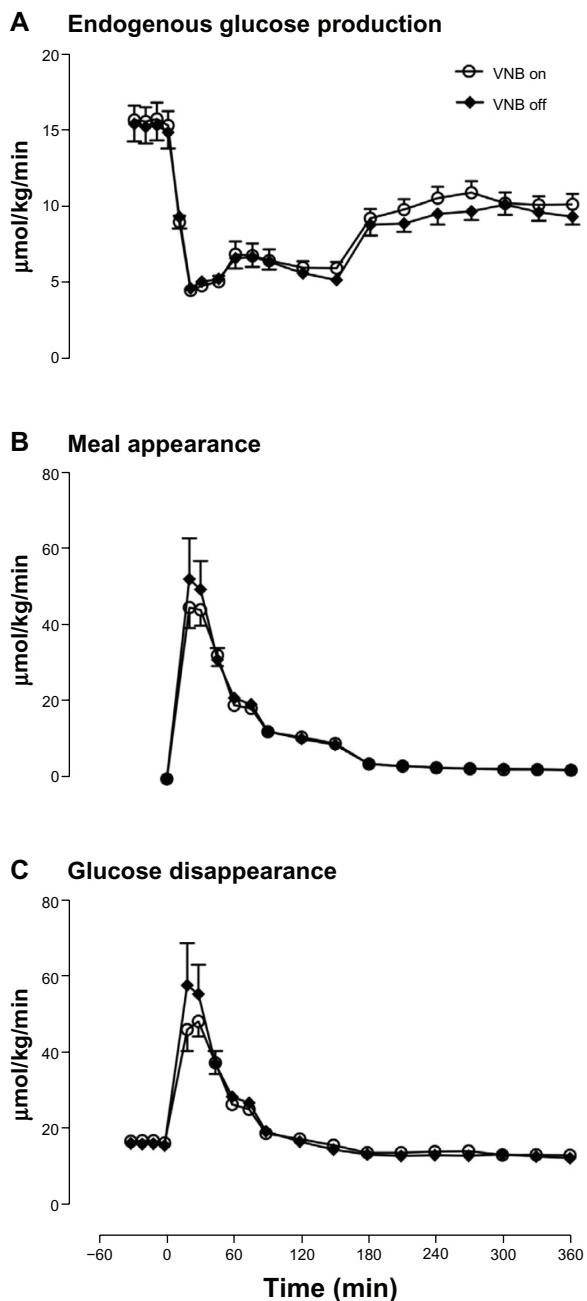
Fasting glucose production (Figure 2A) was unchanged by vagal blockade ( $15.4 \pm 0.9$  vs  $15.2 \pm 1.1$   $\mu\text{mol}/\text{kg}/\text{min}$ ,  $P=0.48$ ). Similarly, postprandial suppression of glucose production by meal ingestion was not altered by vagal blockade ( $4.7 \pm 0.4$  vs  $4.5 \pm 0.3$   $\mu\text{mol}/\text{kg}/\text{min}$ ,  $P=0.14$ ).

The rate of appearance of ingested glucose did not differ in the presence or absence of vagal blockade (Figure 2B) in terms of peak rate of appearance ( $46 \pm 3$  vs  $55 \pm 8$   $\mu\text{mol}/\text{kg}/\text{min}$ ,  $P=0.35$ ), time to peak ( $31 \pm 3$  vs  $28 \pm 4$  minutes,  $P=0.60$ ) and integrated total rate of appearance ( $3,407 \pm 122$  vs  $3,802 \pm 257$   $\mu\text{mol}$  per 6 hours,  $P=0.26$ ).

Postprandial peak rate of glucose disposal (Figure 2C) ( $50 \pm 3$  vs  $60 \pm 8$   $\mu\text{mol}/\text{kg}/\text{min}$ ,  $P=0.24$ ), and integrated rate of postprandial glucose disposal did not differ in the presence or absence of vagal blockade ( $6,315 \pm 137$  vs  $6,601 \pm 304$   $\mu\text{mol}$  per 6 hours,  $P=0.47$ ).

## Insulin action, $\beta$ -cell responsivity, disposition index, and hepatic extraction of insulin in the presence or absence of vagal nerve blockade

Insulin action (Figure 3A:  $7.9 \pm 2.7$  vs  $6.5 \pm 1.2$   $10^{-4}$   $\text{dL}/\text{kg}/\text{min}$  per  $\mu\text{U}/\text{mL}$ ,  $P=0.81$ ),  $\beta$ -cell responsivity ( $\phi_{\text{total}}$ , Figure 3B:  $56 \pm 15$  vs  $47 \pm 8$   $10^{-9}$   $\text{min}^{-1}$ ,  $P=0.58$ ), DI (Figure 3C:  $713 \pm 240$  vs  $532 \pm 145$   $10^{-14}$   $\text{dL}/\text{kg}/\text{min}^2$  per pmol/L,  $P=0.30$ )



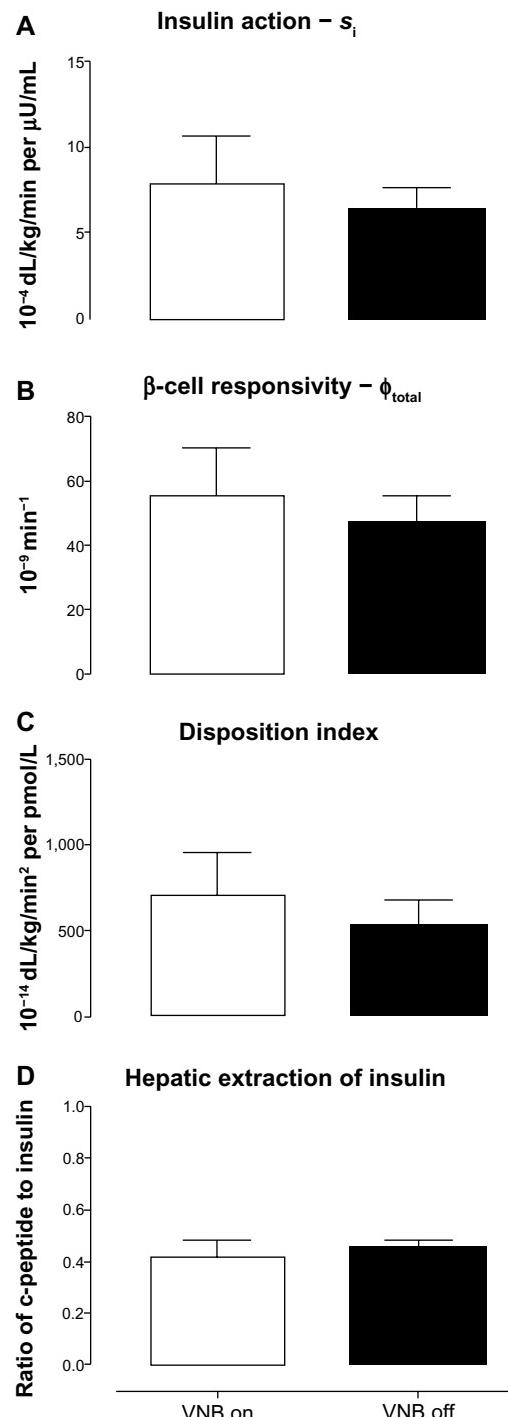
**Figure 2** Endogenous glucose production (A), meal appearance (B), and glucose disappearance (C) in the presence (white open circle) or absence of VNB (solid black diamond).

**Abbreviations:** min, minute; VNB, vagal nerve blockade.

and hepatic extraction of insulin (Figure 3D:  $0.41 \pm 0.06$  vs  $0.45 \pm 0.03$ ,  $P=0.47$ ) did not differ in the presence and absence of VNB.

### Gastric emptying in the presence or absence of vagal nerve blockade

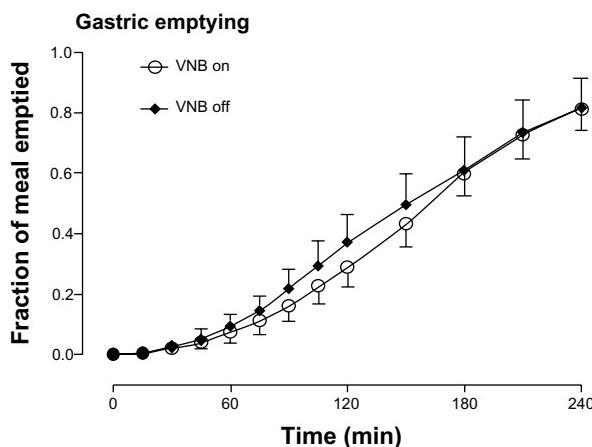
The fraction of the solid meal retained within the stomach did not differ at any time point in the presence or absence of



**Figure 3** Insulin action ( $S_i$ , A),  $\beta$ -cell responsivity ( $\phi_{\text{total}}$ , B), disposition indices (C), and hepatic extraction of insulin (D) concentrations in the presence (white open column) or absence of VNB (solid black column).

**Abbreviations:** min, minute; VNB, vagal nerve blockade.

vagal blockade. Similarly, the time taken to empty 50% of gastric contents ( $160 \pm 15$  vs  $160 \pm 23$  minutes,  $P=0.53$ ) did not differ (Figure 4). Colonic transit at 24 hours was unchanged ( $2.16 \pm 0.38$  vs  $2.39 \pm 0.44$ ,  $P=1.00$ ) in the presence or absence of vagal blockade.



**Figure 4** Gastric emptying in the presence (white open circle) or absence of VNB (solid black diamond).

**Abbreviations:** min, minute; VNB, vagal nerve blockade.

## Discussion

The effect of vagal interruption on glucose metabolism in humans has been an area of uncertainty. We report that VNB, previously shown to reversibly block A $\delta$  and C components of vagal compound action potentials,<sup>31</sup> does not alter fasting and postprandial glucose, insulin, and glucagon concentrations. There were also no effects on fasting and postprandial glucose metabolism. Consequently, we conclude that in obese, nondiabetic humans, vagal blockade has no effect on insulin secretion and action or on glucose metabolism.

The vagus nerve comprises both efferent cholinergic fibers, which mediate secretomotor responses, and afferent fibers that transmit sensory impulses from chemoreceptors and mechanoreceptors distributed throughout the intestine. Preclinical studies in animals have suggested an effect of vagal blockade on both afferent and efferent impulse transmission.<sup>16,31</sup> Of note, surgical interruption of the vagus nerve is usually associated with some degree of remodeling, which can result in reformation of afferent but not efferent pathways.<sup>10</sup> Whether this can explain the diminishing effect of vagal blockade on weight loss over time is, at present, unresolved.<sup>7,8</sup> The participants in this study did not report the presence of bowel symptoms at the time of screening in response to the validated bowel disease questionnaire<sup>20</sup> suggesting some degree of adaptation to vagal blockade. This is consistent with the prior observation of normal gastric accommodation in asymptomatic subjects with chronic diabetic vagal neuropathy.<sup>32</sup>

The absence of an effect of chronic VNB on basal glucose production contrasts with the effect of acute vagal cooling on hepatic glucose production in dogs which produced

an ~20% decrease in fasting glucose production.<sup>15</sup> Our current sample size may have precluded detection of an effect of similar magnitude on glucose production, although we had greater than 80% power to detect a 25% change in fasting EGP. Effects of vagal interruption on canine hepatic glucose metabolism have not been observed in the presence of hyperinsulinemia and hyperglycemia.<sup>33</sup> Such conditions – similar to those during the postprandial period – maximally stimulate hepatic glucose uptake and suppress hepatic glucose production, implying that any potential effect on glucose metabolism is overcome by the effects of postprandial glucose and insulin concentrations. This would be congruent with our observations in the postprandial period. No effect on systemic meal appearance or peripheral glucose disposal was observed. In this experiment, direct measurement of gastric emptying of the solid phase of the meal also did not demonstrate an effect of vagal blockade on solid gastric emptying, again suggesting that the enteric nervous system can compensate for vagotomy or vagal blockade.<sup>10,32</sup>

Our studies were conducted after conclusion of the randomized phase of the original study of the effect of vagal blockade on weight loss, when all subjects were treated with electrical vagal blockade and had been using the activated device for more than a year. It is possible that the same mechanisms that lead to tachyphylaxis after vagotomy obscure any potential effects on glucose metabolism. Given the small sample size available for study, it is conceivable that we were underpowered to detect a small effect of VNB on glucose metabolism. However, in the present experiments it is important to observe the near identical glucose concentrations in the presence or absence of VNB so that with the observed variance in glucose concentrations, >100 subjects would be required to detect a 10% change in glucose concentrations with vagal blockade. Such an effect size would be unlikely to be of clinical significance, especially when compared to other pharmacologic interventions and their effect on glycemic control.<sup>34</sup>

It is also possible that a beneficial effect on glucose metabolism, and more specifically  $\beta$ -cell function, is more difficult to demonstrate in nondiabetic individuals who despite impaired fasting glucose have relatively intact insulin secretory capabilities compared to people with type 2 diabetes,<sup>35</sup> and future studies would need to be conducted in diabetic patients. Indeed, a more recent study in 26 subjects with type 2 diabetes using a similar device and electrical algorithms achieved sustained weight loss over a 1-year period, together with improved HbA<sub>1c</sub>, fasting glucose, and blood pressure.<sup>36</sup> While encouraging, this study did not include a control group.

Moreover, the study design does not allow ascertainment of whether there is a direct effect of vagal blockade on glucose metabolism or whether the metabolic effects are dependent on the weight loss observed in subjects with type 2 diabetes.

We conclude that in the short-term, inactivation of vagal blockade over 1 year after initial implantation has little effect on glucose metabolism and insulin secretion. While this study does not preclude a small effect of vagal blockade on glucose metabolism that is independent of weight loss, such an effect is unlikely to be of clinical significance in nondiabetic subjects.

## Acknowledgments

This work is supported by National Institutes of Health grant numbers DK82396 and DK50456. The authors acknowledge the support of the Mayo Clinic General Clinical Research Center (funded by TR000135). Dr Vella and Dr Cobelli are supported by DK78646 and by DK82396.

## Author contributions

All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

## Disclosure

Dr Vella has received research grants from Merck and Daiichi-Sankyo. He has consulted for Sanofi-Aventis, Novartis, and Bristol-Myers Squibb. Dr Camilleri has served as a consultant for Enteromedics. The other authors report no conflicts of interest in this work.

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